Kidney International, Vol. 48 (1995), pp. 1687–1696

Adhesion molecules in renal disease

Adhesion molecules are a heterogeneous class of ligands/receptors that mediate cell adhesion, either to other cells or to the extracellular matrix. Cell adhesion is of fundamental importance to an impressive number of physiological and pathological processes, including the differentiation of cells and their organization in tissues [1], the intercommunication and activation of immune cells [2], the recirculation and migration of white blood cells [3], the growth and metastatic diffusion of tumoral cells [4]. On the basis of molecular, structural and functional differences, adhesion molecules have been separated into three main groups: the integrins, the selectins and a group that belongs to the immunoglobulin superfamily. In addition to these classic families of adhesion molecules, a recently described family of chemoattractive cytokines, termed chemokines, behave as adhesion molecules after having been released at a site of inflammation. These ligands, in fact, bind to specific receptors in the endothelium or extracellular matrix [5] and here regulate immune cell migration by haptotaxis, a process driven by the gradient of adhesive ligands affixed to the surface of cells or to the extracellular matrix [6].

This article first summarizes the features that distinguish the families of adhesion molecules and gives a concise description of their most relevant members. Then, the expression of adhesion molecules in renal cells in culture and in normal renal tissue, and the pathophysiological role of adhesion molecules in renal disease, with an emphasis on nephritis, transplant rejection and the effects of hemodialysis on leukocytes will be reviewed.

Families of adhesion molecules

Integrin family

The name integrins was originally coined to signify the role of these proteins in integrating the cytoskeleton with the extracellular matrix. Actually, integrins are very versatile and mediate both cell-to-matrix and cell-to-cell adhesion. All integrins are membrane glycoproteins consisting of two subunits, a larger alpha chain and a smaller beta chain [4]. A classification in subfamilies is based on the observation that some members have the same beta chain but different alpha chain. Thus, we distinguish $beta_1$ integrins (also called VLA, very late antigens, because some of them appear on lymphocytes 2 to 4 weeks after antigen stimulation) [7], $beta_2$ integrins (called leukocyte integrins because of their exclusive expression on leukocytes) [2] and $beta_3$ integrins or cytoadhesins [8]. In the last few years, however, new beta chains have been discovered, and it has been found that some alpha subunits are linked to more than one beta subunit, making the original classification insufficient [9].

All integrins conserve a strong sequence homology and structural and functional similarity. Thus, the ligand binding site is formed by short sequences on both chains [1]; the alpha chains have three or four divalent cation-binding motifs [2], and the intracytoplasmic domain interacts with the cytoskeleton through an association with talin and, perhaps, other cytoplasmic proteins. Many, but not all integrins recognize the specific amino-acid sequence RGD (Arg-Gly-Asp) and synthetic peptides with this sequence can block integrin-ligand interaction [2, 9].

The $beta_1$ integrins are formed by the combination of the $beta_1$ chain with different alpha chains and are numbered VLA-1 to VLA-6 according to the alpha chain number (α_1 to α_6). They are expressed on several cell types (leukocytes, platelets, fibroblasts, epithelial cells and endothelial cells) and typically bind to extracellular matrix proteins, mainly laminin, collagen and fibronectin. Thus VLAs promote attachment of endothelial and epithelial cells to substrata [9], adhesion of platelets to exposed subendothelial matrix, tissue repair, and localization of leukocytes in inflamed tissue [4]. Interesting and unusual is the behavior of VLA-4, an integrin expressed on lymphocytes, monocytes, basophils and eosinophils, that functions as a receptor for both fibronectin and VCAM-1, a ligand of the immunoglobulin superfamily expressed on endothelial cells. VLA-4 therefore plays a role in leukocyte-endothelial interactions.

The $beta_2$ integrins consist of three members that are exclusively expressed on leukocytes and are called LFA-1, Mac-1 and p150,95, respectively. LFA-1 is found on virtually all immunocytes, while Mac-1 and p150,95 are predominantly expressed on monocytes and granulocytes. These integrins are constitutively expressed in a nonactive conformation. Cell activation is followed by conformational change of the surface molecule and an increase in the avidity of the receptor, as well as by rapid mobilization from an intracellular pool and increased surface expression [10]. All three $beta_2$ integrins mediate adherence of leukocytes to endothelium, a fundamental step in leukocyte migration. LFA-1 is the best studied and, perhaps, the most important; its endothelial ligands are two proteins of the immunoglobulin superfamily: ICAM-1 and ICAM-2. In addition to endothelial cells, ICAM-1 is induced by activation in several other cell types, including lymphocytes, monocytes, tissue macrophages and epithelial cells. Thus, the interaction of LFA-1 with its counter-receptor ICAM-1 covers a broad range of immune cell contacts and functions including antigen presentation, T-helper and B-lymphocyte responses, natural killing, and adherence of leukocytes to epithelial cells [2].

The $beta_3$ integrins are expressed on platelets, endothelium, polymorphonucleates and monocytes. All bind fibrinogen and von Willebrand factor, and some also bind fibronectin and vitronectin. The member known as gp IIb/IIIa is the major integrin of platelets where it binds soluble fibrinogen and von Willebrand factor. These molecules work as multivalent ligands, that is, they bind to other gp IIb/IIIa receptors on other platelets promoting platelet

Received for publication November 16, 1994
and in revised form January 26, 1995
Accepted for publication January 26, 1995

aggregation, or to receptors on endothelial cells or leukocytes, causing adherence of platelets to these cells.

A number of integrins are formed with newly discovered beta chains and are not as yet grouped in any subfamily. Some of them are expressed on epithelium ($\alpha_6\beta_4$) or on endothelium ($\alpha_v\beta_5$) and play a role in cell attachment to matrix substrate.

Selectin family

The selectins are three surface glycoproteins characterized by a similar structure consisting of an N-terminal lectin-like domain, an epidermal growth factor (EGF) repeat, and a variable number of modules homologous to complement binding proteins [11]. The term selectin highlights the functional importance of the lectin domain and the selectivity of distribution and function of these molecules. The lectin domain plays a crucial role, of course, containing the site that binds to specific carbohydrate counter-receptors, but the EGF domain is likewise essential in mediating adhesion. The role of the complement binding-like modules is less defined, possibly involving the mechanical support that keeps the lectin and EGF domains away from the cell surface. The selectins are named according to the cell type on which they were originally identified: E-selectin (endothelial cells), P-selectin (platelets) and L-selectin (leukocytes). E-selectin expression is restricted to endothelial cells activated by endotoxin or cytokines [12]. P-selectin is found in both platelets and endothelial cells, where it is stored in alpha and Weibel-Palade granules, respectively, in the resting state [13], and is rapidly redistributed to the cell surface upon activation by thrombin, histamine or other mediators [14]. L-selectin is constitutively expressed on lymphocytes, neutrophils and monocytes and is rapidly shed from their surface after cell activation. All three selectins bind to one or more carbohydrate ligands, mainly to sialyl-Lewis x and other fucosylated carbohydrates [15]. Counter-receptors of E-selectin are found on granulocytes, monocytes and a subpopulation of memory T-lymphocytes; P-selectin ligand is similarly distributed on leukocytes, and L-selectin ligand is expressed on endothelial cells [15]. Thus, all three selectins support the adhesion of leukocytes to endothelium, a necessary step for leukocyte migration in inflamed tissue. The adhesive reaction mediated by selectins is very quick, involving small carbohydrate epitopes [16], and is of low affinity. It is now clear that selectins play a role in the first step of leukocyte migration, which consists of leukocyte rolling on the endothelial surface and precedes the firm adherence mediated by integrins [17]. In addition to being essential for leukocyte recruitment in inflamed tissue, selectins regulate lymphocyte recirculation, that is, the passage of lymphocytes from blood to tissue or lymph node and then back to blood. Thus L-selectin functions as a "homing receptor" (this term refers to receptors on lymphocytes, while the counter-receptors on endothelium are called "addressins") for peripheral lymph nodes, while E-selectin functions as addressin for a subtype of memory lymphocytes [3].

Immunoglobulin superfamily

As previously discussed, the adhesion molecules of this group are ligands of integrin receptors.

ICAM-1 and ICAM-2 (intercellular adhesion molecule-1 and -2), the counter-receptors of $beta_2$ integrins, are closely related in structure and function but differ in the number of Ig domains (5 and 2, respectively). ICAM-2 is constitutively expressed on endothelial cells and its expression is not modified by stimulation; in contrast, the constitutive expression of ICAM-1 on endothelial cells varies in different vascular districts and is usually low. Endothelial expression of ICAM-1, however, increases several times after cell activation with IL-1, TNF or γ-interferon [18]. In addition to endothelial cells, other cell types express ICAM-1 after exposure to inflammatory stimuli, including leukocytes, epithelial cells, dendritic cells, and fibroblasts. Thus the interaction between $beta_2$ integrins (mainly LFA-1) and ICAM-1 regulates leukocyte migration, the immune responses that depend on adhesive contact between immune cells, and the binding of leukocytes to epithelial cells and fibroblasts [2]. Circulating forms of ICAM-1 were recently detected and their biological functions are currently under investigation [19].

VCAM-1 (vascular cell adhesion molecule-1) is another adhesion molecule of the immunoglobulin superfamily that contains seven Ig domains. VCAM-1 is induced by activation on endothelial cells and supports the adhesion to endothelium of memory lymphocytes [20] and other leukocytes expressing its $beta_1$ integrin counter-receptor VLA-4, that is, monocytes, basophils and eosinophils. VCAM-1 is also expressed on several non-vascular cell types, including dendritic cells in lymph node and skin, bone marrow stromal cells and synovial cells [11].

Chemokines

The chemokines are a superfamily of polypeptides that are characterized by the presence of four conserved cysteine (C) residues. Two subfamilies are distinguished according to whether another amino acid (X) is interposed between the first two C residues (alpha or C-X-C subfamily), or whether these two C residues are adjacent (beta or C-C subfamily). At present, fifteen related chemokines have been described that are secreted by different cell types such as platelets, white blood cells, endothelial and epithelial cells, macrophages, and fibroblasts [21]. All chemokines are chemoattractants, that is, they attract selected cell types expressing receptors specific for them. Representative members of the two subfamilies are interleukin-8 (C-X-C type) and RANTES (C-C type), both of which play a relevant role in inflammation by participating in leukocyte recruitment. These chemokines function through a mechanism known as haptotaxis, which makes them more similar to adhesion molecules than to classic chemotactic factors. In fact, they do not diffuse freely away from their site of production but bind to activated endothelium or extracellular matrix, so that leukocyte migration occurs along the gradient of chemokine affixed to the substratum. Leukocyte receptors for chemokines are members of the G-protein-coupled receptors and not only direct migration, but also activate integrin adhesiveness and stimulate degranulation and the respiratory burst. Their cellular distribution determines the responding cell; thus, the IL-8 receptor is restricted to neutrophils, while the RANTES receptor is expressed on monocytes, basophils, eosinophils, and a subset of T lymphocytes with high reactivity to recall antigens [22].

Renal expression of adhesion molecules

Adhesion molecules in cultured renal cells

Glomerular epithelial cells. Rat glomerular epithelial cells (GEC) in culture constitutively express the $beta_1$ integrins VLA-2 and VLA-3. These integrins mediate GEC adhesion to plastic wells coated with extracellular matrix proteins (collagen I and IV, laminin and fibronectin), and the adhesion is divalent cation dependent, a function characteristic of integrins [23]. These

observations suggest that $beta_1$ integrins regulate *in vivo* GEC attachment to glomerular basement membrane and play a role in maintaining the normal architecture of the glomerular capillary wall. It is of interest that the adhesion of rat GEC to collagen induces responsiveness to epithelial growth factors and activates phospholipase C [24], suggesting that the interaction of integrins with extracellular matrix regulates not only adhesion, but also GEC proliferation. Caution should be used, however, when extrapolating these data to the *in vivo* condition, in which GEC assume a unique shape with only the foot processes left in contact with the basement membrane. Recent studies, in fact, have shown that VLA-2 is not espressed in normal rat glomeruli, nor in GEC newly isolated from glomeruli, but is synthesized *ex novo* by GEC after several passages in culture, a phenomenon that probably depends on loss of differentiation [25]. Accordingly, immunoprecipitation of GEC lysates with anti-alpha chain antibodies has revealed only α_3, that is, the alpha chain of VLA-3 [26]. VLA_3 therefore appears to be the major or sole GEC integrin receptor.

Glomerular endothelial cells. In vitro rat glomerular endothelial cells express $beta_1$ integrins that mediate adhesion to components of the glomerular basement membrane; in particular, in culture conditions VLA-5 is the major fibronectin receptor, VLA-3 binds type I collagen, and VLA-1 participates in adhesion to laminin [27].

Mesangial cells. Human mesangial cells in culture use the VLA-5 integrin receptor to bind to wells and beads coated with fibronectin; interestingly, this VLA-5-mediated adhesion activates phagocytosis of fibronectin-coated beads. [28]. Recently, mRNA transcripts of integrin alpha chains 1, 2, 3 and 5 have been found in cultured human and rat mesangial cells, and immunofluorescence analysis has revealed that their protein products are concentrated into focal adhesions, supporting their functional relevance [29].

Murine mesangial cells express low constitutive levels of ICAM-1 and ICAM-1 mRNA transcripts. The expression of ICAM-1 increases markedly after stimulation with INF-γ and TNF, and mesangial cells become capable of adhering to T lymphocytes and of presenting antigen; anti-ICAM-1 mAb inhibits both adhesion and antigen presentation [30].

Tubular cells. $Beta_1$ integrins are expressed in primary cultures of human renal tubular epithelial cells and contribute to cell attachment to substratum; more precisely, VLA-5 binds tubular cells to fibronectin, and VLA-2 and VLA-6 bind them to laminin [31]. In addition to the ventral surface of cells, VLA-2 has also been detected at intercellular borders, where it may function during cell-cell interaction [32].

ICAM-1 was found to be constitutively expressed in over 90% of human proximal tubular cells in an established primary culture. ICAM-1 expression was up-regulated by supernatants from mixed lymphocyte reaction and by recombinant cytokines, especially INF-γ, TNF-α and IL-1 [33]. Adhesion of activated T lymphocytes to human tubular cells *in vitro* is inhibited by antibody to ICAM-1 [34]. These findings suggest that ICAM-1 plays a critical role in mediating lymphocyte adhesion to tubular cells.

VCAM-1 and its mRNA transcript are expressed in unstimulated human tubular cells in primary culture, and their expression is enhanced by stimulation with TNF-α [35]. Similar constitutive VCAM-1 expression and up-regulation by cytokines occurs in primary culture of mouse tubular cells; adhesion of homologous T cells and monocytes layered on stimulated murine tubular cells is inhibited by anti-VCAM-1 antibody [36].

Table 1. Expression of adhesion molecules in normal human kidney

Adhesion molecule	Renal expression		
	Glomeruli	Tubules	Interstitium and vessels
Integrins			
VLA-1	Mesangium, Bowman's capsule	All tubular cells	NE
VLA-2	Endothelium	Distal tubule	NE
VLA-3	Mesangium Visceral epithelium Parietal epithelium Endothelium	Distal tubule	NE
VLA-5	Mesangium Endothelium	NE	NE
VLA-6	NE	All tubules	NE
LFA-1	Monocyte/macrophage	NE	Monocyte/ macrophage
Ig superfamily			
ICAM-1	Endothelium	NE	Vascular endothelium
VCAM-1	Parietal epithelium	Proximal tubule	NE
Selectins			
E-selectin	NE	NE	Peritubular capillaries

NE is not expressed.

RANTES mRNA transcript and its protein product are expressed in a culture line of proximal tubular cells of murine origin, and the transcript level rises in response to stimulation with TNF-α and IL-1α [37]. IL-8 has also been detected in human renal epithelium [38]. These observations suggest that chemokines released *in vivo* by inflammation-damaged tubular epithelial cells may provoke or enhance the infiltration of leukocytes in renal interstitium.

Adhesion molecules in normal kidney

The expression of adhesion molecules in normal human kidney is summarized in Table 1.

Integrins. With the help of immunoelectron microscopy a fibronectin receptor of the integrin $beta_1$ subfamily was localized early in human kidney, in the membranes of glomerular cells facing the mesangial matrix and the glomerular basement membrane [39]. Successive studies using monoclonal antibodies specific to single $beta_1$ integrins have identified VLA-3 (a receptor that binds fibronectin, laminin and collagen) as the predominant VLA in the human glomerulus, where it is located in the mesangium, Bowman's capsule and on the visceral epithelial and endothelial cell surfaces in contact with the basement membrane. The predominantly basal distribution of VLA-3 suggests that it has a role in the attachment of glomerular endothelial and visceral epithelial cells to glomerular basement membrane [28]. In other studies, VLA-1 (receptor for laminin and collagen) was detected in mesangium and Bowman's capsule, VLA-2 (receptor for laminin) in glomerular endothelial cells, and VLA-5 (receptor for fibronectin) in mesangium and glomerular endothelial cells [32, 40]. $Beta_1$ integrins have also been isolated in proximal tubular cells, where they are strictly basally confined, and in distal tubule and ascending Henle's loop, where they are distributed both in a sharp linear fashion basally and diffusely throughout the rest of the cell membrane [41]. MoAbs directed against specific alpha chains have demonstrated that the $alpha_6$ chain, along with its

corresponding beta$_1$ integrin VLA-6 (laminin receptor), is confined basally in all renal tubules, presumably anchoring epithelial cells to the tubular basement membrane [42, 43], while VLA-2 and VLA-3 are restricted to the distal nephron [32]. VLA-1 (collagen receptor) is also expressed on the basal aspect of all tubular cells [44].

In normal kidneys LFA-1 expression is confined to isolated cells throughout the interstitium and in the glomeruli. In the latter, LFA-1 expressing cells number 1 to 10 per section and have been identified by immunostaining with specific antibodies as monocytes or migrating macrophages [34].

Immunoglobulin superfamily. The expression of ICAM-1 in normal human kidney has been the object of several studies [34, 35, 45, 46]. All agree that ICAM-1 is constitutively expressed in renal vascular endothelium, usually more intensely in interstitial capillaries and venules than in arterial vessels and glomerular capillaries. Most investigators have found no evidence of ICAM-1 expression in the mesangium, visceral glomerular epithelium, renal tubules; however, weak mesangial expression has also been described [47].

VCAM-1 is normally expressed on some parietal epithelial cells in Bowman's capsule and parts of the proximal tubule, but it is not constitutively expressed on visceral epithelium or endothelium [35, 48]. It is interesting that VCAM-1 distribution in normal kidney is complementary to that of the other immunoglobulin ligand of integrin receptors, ICAM-1, suggesting some complementation of roles in renal pathophysiological conditions characterized by the engagement of leukocytes.

Selectins. It has been reported that E-selectin is not detectable in normal human kidney [35], or that it is occasionally expressed in isolated peritubular capillaries [49]. The carbohydrate ligand of L-selectin, sLe^x, has been shown in small amounts in normal rat glomeruli [50].

Chemokines. The only study on distribution of RANTES in "normal" human kidney has been performed in renal biopsy samples of transplanted kidneys taken one hour after vascular anastomosis during transplantation surgery; in these kidneys neither RANTES nor its mRNA transcript were expressed [51].

Table 2. Adhesion molecules in renal disease

Adhesion molecules	Changes in renal disease (interested cell or site)
Intergrins	
β_1 integrins	δ Heymann nephritis (podocyte)
	δ "new" models of NTN (podocyte)
	δ anti-thymocyte serum nephritis (glomeruli)
	δ ischemic/toxic acute renal failure (tubular cells)
	↓ **membranous nephropathy** (glomerular capillary)
	↑ **IgA nephropathy** (mesangium)
β_2 integrins	↑ **hemodialysis** (peripheral leukocytes)
α_3, α_6, α_v subunits	δ **renal carcinoma** (tumoral cells)
Ig superfamily	
ICAM-1	↑ "classic" NTN (glomerular and interstitial capillary endothelium, interstitial resident cells)
	↑ murine lupus nephritis (mesangium, proximal tubule, vascular endothelium)
	↑ **focal glomerulosclerosis** (mesangium)
	↑ **extracapillary glomerulonephritis** (cellular crescents, tubules)
	↑ **human lupus nephritis** (glomeruli, tubules)
	↓ **membranous nephropathy** (glomerular capillary endothelium)
	↑ **"active" nephritides** (tubules)
	↑ **acute and chronic rejection** (tubules)
	↓ **hemodialysis** (peripheral leukocytes)
VCAM-1	↑ "classic" NTN (glomerular capillary endothelium)
	↑ murine lupus nephritis (mesangium, proximal tubule)
	↑ **glomerulonephritides and noninflammatory glomerular diseases (diabetes, gout and amyloid nephropathy)** (proximal tubule)
	↑ **acute rejection** (vascular endothelium, tubules, dendritic interstitial cells)
	↑ **cryoglobulinemia** (glomerular capillary wall)
Chemokines	
RANTES	↑ **acute rejection** (tubular cells, peritubular capillary endothelium)
Selectins	
E-selectin	↑ "classic" NTN (glomerular capillary endothelium)
L-selectin	↓ **hemodialysis** (peripheral leukocytes)

Symbols are: ↑ upregulation, ↓ downregulation, δ dysfunction. Changes found in human diseases are indicated in bold.

Pathogenic role of adhesion molecules in renal disease

The changes in adhesion molecules associated with renal disease are summarized in Table 2.

Adhesion molecules in nephritis

Experimental models: Heymann nephritis and nephrotoxic serum nephritis. In classic passive Heymann nephritis (HN), proteinuria occurs when anti-Fx1A antibodies raised against a proximal tubular antigen cross react with antigens on glomerular visceral epithelial cells (GEC) and gradually accumulate in the subepithelial space, leading to activation of complement and proteinuria in four to five days [52]. Proteinuria, however, can also be induced in 24 hours by injecting F(ab)$_2$ or F(ab)′ fragments of anti-Fx1A antibody, which do not activate complement [53]. This rapid-onset, complement-independent proteinuria is transient and associated with fine subepithelial deposits and a characteristic morphologic alteration consisting of effacement of GEC foot processes. Clearly the lesion produced by F(ab) fragments differs from that of classic HN. Adler and Chen recently showed that anti-Fx1A antibody recognizes the VLA-3 integrin receptor on GEC in culture. In their study, anti-Fx1A inhibited the adhesion of GEC to several substrata and produced reversible cell detachment and "rounding up" when added to adherent cells [54]. These results suggest that the binding of anti-Fx1A or its F(ab) fragments to the main integrin receptor of podocytes may alter the normal adhesion of GEC to GBM and cause cell detachment, which is responsible for altered glomerular permeability and proteinuria. The different disease courses caused by whole antibody and F(ab) fragments depend on differences in size and ability to activate complement. Due to their small size, F(ab) fragments are able to accumulate rapidly in the subepithelial space and in a short time produce a transient proteinuria, transient because it is not sustained by the inflammatory cascade initiated by complement activation.

Nephrotoxic serum nephritis (NTN) is a model of nephritis induced in rat by injecting heterologous anti-kidney antiserum. In classic NTN renal injury is both complement- and leukocyte-dependent and is caused by antibodies directed against antigens in the GBM. In the last few years, however, it has become clear that

nephrotoxic sera are polyreactive, that is, they contain antibodies directed against several cell-surface antigens in addition to the classic GBM antigens [55]. Furthermore, it has been shown that some of these antibodies can cause proteinuria directly, in the absence of complement activation or inflammatory cell infiltrates. Several new models of NTN have thus been designed [56, 57] that are clearly different from classic inflammatory NTN. In these models of NTN a characteristic morphological correlate of proteinuria is effacement and detachment of podocytes from GBM, so that it is reasonable to speculate that antibodies interfere with normal podocyte-GBM adhesion. Early evidence that podocytes might be the target of antiserum in complement-independent NTN was given by the demonstration that a monoclonal antibody against the epithelial surface antigen SGP-115/107 can directly induce both proteinuria and the characteristic structural alterations [58]. Recently, O'Meara and coworkers have shown that a non complement-binding anti-rat nephrotoxic serum reacts with surface antigens of GEC. This antiserum immunoprecipitates two proteins from GEC that are co-precipitated by a monoclonal antibody identifying the $beta_1$ integrin receptor for fibronectin. In addition, in culture the antiserum inhibits adhesion of GEC to collagen, laminin and fibronectin, and prevents GEC spreading on a variety of matrix proteins [59]. These findings suggest that anti-integrin antibodies disrupt GEC anchorage to GBM and thus cause detachment of foot processes and alteration of glomerular capillary permeability.

In summary, modifications of two classic models of nephritis have led to the development of models of antibody-dependent, complement-independent renal injury characterized by rapid-onset proteinuria, alterations of podocyte morphology (effacement of foot processes), and detachment of epithelial cells from the basement membrane. Interference with the normal anchorage of GEC to GBM caused by antibodies to integrin receptors on podocytes seems to be the common pathogenic mechanism in these models. It is reasonable to speculate that similar interferences with integrin function, caused by toxic substances or circulating factors, may play a pathogenic role in other models of non-inflammatory proteinuria, for example, puromycin nephrosis or human minimal change nephropathy.

Recent studies have shown that leukocyte adhesion molecules and their endothelial ligands also play a fundamental pathogenic role in classic NTN caused by anti-GBM antibodies. Infusion of anti-GBM antibodies, in fact, is followed by up-regulation of E-selectin, ICAM-1 and VCAM-1 on glomerular capillary endothelium. Leukocyte recruitment in glomeruli and proteinuria is inhibited by treating rats with antibodies against ICAM-1 and VCAM-1, and their counter-receptors LFA-1, Mac-1 and VLA-4. Protection is also afforded by anti-TNF-α antibodies, suggesting that this cytokine, perhaps released locally, is responsible for up-regulation of endothelial adhesion molecules [60–62]. In the early phase of anti-GBM nephritis, ICAM-1 is strongly up-regulated in the endothelium of interstitial capillaries as well and is expressed *de novo* in interstitial resident cells. ICAM-1 therefore appears to direct migration and localization of interstitial leukocytes [63].

Other models of nephritis. Wuthrich et al studied the renal expression of ICAM-1 and VCAM-1 in murine models of lupus nephritis. With the aid of immunoperoxidase staining they showed a strong up-regulation of ICAM-1, in particular in the brush border of proximal tubules, in the mesangium and in the endothelium of large vessels. Northern analysis revealed a two- to fivefold increase in the levels of ICAM-1 transcripts in the kidney of nephritic mice [64]. Like ICAM-1, VCAM-1 was up-regulated in cortical tubular and mesangial cells, but the tubular expression of VCAM-1 was more focal, being localized in tubules adjacent to mononuclear infiltrates. Adherence assays on kidney sections from autoimmune rats showed an increased adhesiveness of T cell and macrophage cell lines and of lymph node cells that was inhibited by monoclonal antibodies targeting the ICAM-1 and VCAM-1 molecules [36]. These results indicate that enhanced ICAM-1 and VCAM-1 expression confers increased adhesiveness on the renal parenchyma in lupus nephritis and serves as a pathway by which inflammatory cells adhere to renal tissue and promote renal injury.

Injection of anti-thymocyte serum produces an acute form of glomerulonephritis in the rat characterized by transient mesangiolysis, followed by mesangial cell activation and mesangial matrix synthesis that is promoted by increased local activity of TGF-β [65]. Recent studies have shown that the increased matrix deposition in this glomerulonephritis is associated with over-expression of glomerular $beta_1$ integrins VLA-1 and VLA-5 and reduced expression of VLA-3. In *in vitro* studies, normal glomeruli treated with TGF-β showed changes in integrin expression mimicking those occurring *in vivo*. These results suggest that the proliferative effect of TGF-β is mediated by altered $beta_1$ integrin expression [66].

Human nephritis. Seron et al studied the expression of VCAM-1 in renal biopsies from patients with interstitial nephritis due to NSAID, systemic vasculitis with crescentic nephritis, and other forms of inflammatory and non-inflammatory glomerular disease (IgA, membranous, minimal change, diabetes, lupus, gout and amyloid nephropathy). Proximal tubular expression of VCAM-1 was elevated in all such disorders, but the increase was especially marked in vasculitis and interstitial nephritis. VCAM-1 expression was positively correlated with the number of activated (transferrin-receptor positive) leukocytes infiltrating the interstitium. Surprisingly, VCAM-1 was never identified on vascular endothelium [48]. Up-regulation of VCAM-1 was also found in different forms of glomerulonephritis by Bruijn and Dinklo [67]. These authors documented increased expression on proximal tubules and on the endothelium of large interstitial vessels, but not on interstitial capillaries, in all the diseases studied (membranous and membranoproliferative glomerulonephritis, IgA nephropathy, lupus nephritis, cryoglobulinemia, Wegener's disease). Particularly strong expression of VCAM-1 in the glomerular capillary wall was observed in cryoglobulinemia. These findings suggest that VCAM-1 plays a pathogenic role in several renal diseases by promoting an interaction between inflammatory and tubular epithelial cells. Such interaction may be necessary for the mononuclear cell cytotoxic attack, as well as for the tubular cell accessory function as antigen presenting cell.

The author and his coworkers have studied the expression of ICAM-1 in primary focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), necrotizing glomerulonephritis (NGN) with crescent formation, and lupus nephropathy. We have shown intense *de novo* expression of ICAM-1 on mesangial cells in FSGS, with focal and segmental distribution, but no up-regulation of the molecule in MN [43]. While the pathogenic role of ICAM-1 in FSGS remains to be established, the increased mesangial expression of this molecule clearly indicates activation of mesangial cells and suggests an inflammatory step in the progression of

the disease. Interestingly, we recently found that ICAM-1 is expressed *de novo* in the mesangium of patients with minimal change disease, similar to what occurs in patients with FSGS (Dal Canton and Meyrier, unpublished observations), which supports the contention that the two diseases are but two sides of the same coin. We also discovered abnormal expression of ICAM-1 in the glomeruli of patients with crescentic glomerulonephritis. In fact, ICAM-1 was strongly expressed in cellular crescents, a finding that suggests a role for this molecule in recruiting macrophages in the early stage of the disease [68]. Increased glomerular expression was a marker of activity in lupus nephritis [69]. Other studies have shown altered renal expression of ICAM-1 in different forms of glomerulonephritis. Lhotta et al observed increased glomerular expression in early cases of rapidly progressive glomerulonephritis and in lupus nephritis, while the expression was reduced in advanced rapidly progressive glomerulonephritis and membranous nephropathy. Increased ICAM-1 expression was also detected on tubular cells in rapidly progressive glomerulonephritis and membranoproliferative glomerulonephritis [45]. Tubular expression of ICAM-1 was studied by Chow et al in renal biopsy specimens from a range of non-immune renal diseases and glomerulonephritides. Increased tubular expression of ICAM-1 was found on undamaged tubules in glomerulonephritis and this showed a strong correlation with disease activity [70]. In summary, ICAM-1 seems to play a role in promoting glomerular recruitment of leukocytes and extracapillary proliferation in exudative and crescentic glomerulonephritis, respectively; in addition, in several other forms of nephritis, *de novo* expression of ICAM-1 on tubular cells confers them a stickiness for adhesive contact with infiltrating leukocytes whose toxic attack may thus be facilitated.

Baraldi and coworkers studied the expression of integrin VLA-3 in renal biopsy specimens from patients with nephrotic syndrome due to membranous nephropathy (MN), minimal change disease and lupus nephritis [71]. It is noteworthy that the normal linear distribution of VLA-3 along the glomerular capillary loop was altered in membranous nephropathy but not in the other diseases, indicating that changes in MN were not an aspecific effect of proteinuria. In stages I and II of MN, VLA-3 distribution showed an irregular, trabecular pattern; in addition, in stage III a segmental loss of VLA-3 was detected [71]. These observations suggest that in human MN, as in its experimental counterpart (Heymann nephritis), a disruption of the normal interaction between VLA-3 and its GBM ligand occurs.

Recently, the expression of fibronectin receptor (assumed to be an integrin) was found to be elevated in the mesangium of patients with IgA nephropathy. This increase was correlated with the degree of histological glomerular damage and proteinuria [72]. The pathophysiological relevance of this observation is not clear, but one may speculate that up-regulation of the fibronectin receptor plays a role in the activation of mesangial cells that is the hallmark of this disease.

Adhesion molecules in renal transplant

Pathogenic role of adhesion molecules in graft rejection. Cell-mediated rejection is an immune reaction characterized by the migration of mononuclear cells into renal tissue and by an effector response driven by the interaction between MHC molecules on donor cells and TCR on recipient T cells. Since both adherence of leukocytes to endothelium (which is crucial for leukocyte migration) and stabilization of the TCR-antigen interaction (necessary for full T cell activation) are mediated by adhesion molecules, it is logical to expect that adhesion molecules play an important pathogenic role in rejection. Until now the interest of investigators has been mainly limited to evaluating renal expression of ICAM-1 in acute and chronic rejection [34, 35, 46, 49, 73–75]. All agree that graft rejection is accompanied by *de novo* expression of ICAM-1 on renal tubular cells. Such expression is predominant in the proximal tubules and has a focal distribution occurring in 15 to 40% of tubules. It is paralleled by up-regulation of HLA class II antigens and is roughly proportional to the level of leukocyte infiltration. The *de novo* tubular expression of ICAM-1, however, is not specific to graft rejection, since it has also been found in non-rejecting kidneys [49] and may depend on ischemic damage during organ retrieval [46]. Increased ICAM-1 expression on renal vascular endothelium has occasionally been described during rejection [46], but this finding is inconsistent, mainly because ICAM-1 is constitutively and diffusely expressed on normal renal endothelium as well [34, 49]. In summary, these studies suggest that in renal graft rejection significant *de novo* ICAM-1 expression on tubules supports the contact between tubular cells and infiltrating leukocytes. In this way, both the antigen presentation by tubular cells (in the context of class II MHC molecules) and the cytotoxic attack of effector cells are facilitated. A role for ICAM-1 in the recruitment of leukocytes into renal tissue has not definitely been proven, and renal expression of ICAM-1 cannot be used to discriminate rejection from other causes of acute graft dysfunction. An attempt has been made to utilize serum levels of circulating ICAM-1 or ICAM-1 expression on renal tubular cells present in urinary sediment as diagnostic indicators of acute rejection, but with poor success [75, 76]. In fact, circulating ICAM-1 concentration increases not only during rejection but also during cytomegalovirus infection, and cells expressing ICAM-1 are found in the urine of both rejecting and non-rejecting patients.

A number of studies have recently investigated the expression of other adhesion molecules in renal graft rejection. In acute rejection *de novo* expression of VCAM-1 is induced on renal vascular endothelium, and the expression of this molecule is up-regulated in the tubules [35, 49, 73, 74]. *De novo* VCAM-1 expression is particularly pronounced on the endothelium of muscular arteries showing features of acute vascular rejection, that is, endothelial separation from the underlying intima and subendothelial infiltration of mononuclear cells. In acute vascular rejection VCAM-1 is also found on a distinct population of dendritic cells within interstitial lymphoid aggregates, and focally on mesangial cells [77]. These results suggest that VCAM-1 plays a double pathogenic role in renal graft rejection by (a) potentiating the interaction between tubular cells and effector cells, and (b) promoting leukocyte migration into renal tissue. *De novo* induced expression on renal endothelium is particularly relevant and seems to be a distinctive feature of rejection that is useful for differentiating acute rejection from other causes of graft dysfunction, for example, cyclosporine toxicity [73].

Only a few studies have examined E-selectin in renal allograft rejection in humans, and no change in its expression was detected [49, 74]. E-selectin, however, is induced early and transiently in inflammatory response so that grafts may have been biopsied after the level of E-selectin had declined. A role for L-selectin and its sialyl Lewis^x ligand in determining the site of lymphocyte extravasation into the graft has recently been shown in a rat model of acute renal graft rejection [50].

The role of the RANTES chemokine in cell-mediated transplant rejection was recently defined in elegant studies by Pattison et al [49]. These authors demonstrated that during rejection both RANTES and RANTES mRNA are expressed by infiltrating mononuclear cells and by renal tubular cells. In addition, they localized RANTES protein (but not mRNA) on the endothelium of peritubular capillaries and showed that RANTES receptors are expressed on this endothelium. These results allow construction of a model of the central role played by RANTES in transplant rejection. Macrophages in the graft produce cytokines that activate the expression of RANTES by renal tubular epithelium. RANTES released by tubular cells diffuses locally and binds to receptors on endothelial cells. After being planted on the endothelium RANTES works as an adhesion molecule, attracting T cells and monocytes to the site of inflammation, and once these cells have entered the interstitium they are driven to their target by the RANTES gradient expressed on tubular cells.

Anti-rejection therapy with anti-adhesion molecule mAbs. An anti-LFA-1 monoclonal antibody was first used successfully to prevent graft failure in children with HLA-mismatched bone marrow transplants [78]. The same antibody (murine mAb 25-3) was then used to treat seven subjects with acute renal graft rejection. Only one patient, who had the lowest increase in blood creatinine, reverted to pre-rejection graft function with 25-3 treatment alone. All the others required additional rescue therapy, so it was concluded that anti-LFA-1 is inefficient in reversing acute ongoing renal rejection. Treatment with 25-3 caused a reversible side effect (Quincke's edema) in only one patient and did not raise anti-murine antibodies in any case [79]. These results, while disappointing, do not exclude the possibility that the antibody may be more useful for preventing renal graft rejection (as in bone marrow transplantation) than for treating rejection episodes in course.

The effects of blocking ICAM-1 were first studied by Cosimi et al in cynomolgus monkeys [80]. These authors administered a murine anti-ICAM-1 mAb (R6.5) as the sole agent to prevent rejection in a group of animals and used R6.5 to treat ongoing acute rejection in another group maintained on cyclosporine. Treatment with the antibody significantly prolonged survival in the first group and reversed rejection in all animals of the second group. These results encouraged Haug et al to use the same antibody in a phase I trial in human renal allograft recipients. Eighteen patients who received allografts at high risk for delayed graft function received doses of R6.5 in order to maintain adequate serum levels (>10 μg/ml) for a two-week course. The patients treated with anti-ICAM-1 mAb had significantly fewer delayed graft function and rejection episodes than a group of patients who received the contralateral kidney from the same donor and conventional treatment. In addition, none of the anti-ICAM-1 treated patients developed primary non-function, in contrast with 3 out of 18 in the control group [81]. Therapy with R6.5 was well tolerated.

In conclusion, preliminary results indicate that anti-ICAM-1, but not anti-LFA-1 therapy, may be useful in treating acute renal graft rejection. Why blocking ICAM-1 is efficient but blocking its ligand is not remains unclear. A possible explanation is that anti-LFA-1 operates only on leukocytes, while anti-ICAM-1 can also act at the target cell level. Part of the benefit displayed by anti-ICAM-1, however, may result from a protective effect on reperfusion injury.

Effects of extracorporeal hemodialysis on leukocyte adhesion molecules

Hemodialysis causes a profound, transient neutropenia whose possible mechanisms include leukocyte aggregation, adhesion to endothelia, and pulmonary sequestration [82]. There is evidence that dialysis-related neutropenia depends on complement activation and generation of the complement fragments C3a and C5a, which can activate leukocytes [83]. Arnaout et al first demonstrated a rapid up-regulation of the granulocyte $beta_2$ integrin Mac-1 during dialysis with cellulose filters [84]. Interestingly, the time course of increased expression of this receptor coincided with the development of granulocytopenia and with the peak rise in plasma levels of the complement activation products C5a desArg and C3a desArg. C5a desArg (but not C3a desArg) induced a comparable increase in Mac-1 expression on normal granulocytes *in vitro* at concentrations similar to those measured *in vivo*. The overexpression of Mac-1 during cuprophane dialysis was confirmed by Alvarez et al, who also found that dialysis increased leukocyte expression of p 150,95, another $beta_2$ integrin, and down-regulated the expression of L-selectin. Similar phenotypic changes were reproduced *in vitro* by incubating neutrophils with activating agents. Kinetic studies showed that both the peak increase in integrin expression and the maximal drop in L-selectin expression occurred after 15 minutes of dialysis, coincident with the nadir in neutrophil count. Neither neutropenia nor the changes in leukocyte adhesion molecules took place when hemodialysis was performed with polyacrylonitrile membranes [82]. The ability of cuprophane membranes to activate serum directly was proved by Lundahl, Hed and Jacobson, who showed that serum preincubated with fragments of cuprophane membranes increased Mac-1 expression on normal leukocytes [85]. The causal relation between complement activation and changes in leukocyte adhesion molecules during hemodialysis was further confirmed by Himmelfarb et al [86], who utilized either first-use cellulose dialyzers (that activate complement) or reused dialyzers (that have minimal complement activating potential). First use dialyzers, but not the reused ones, caused a rapid, fourfold increase in Mac-1 and a twofold decrease in L-selectin. Stimulation of granulocytes *in vitro* with C5a reproduced the same changes seen *in vivo*. Taken together, these results demonstrate that dialysis with cellulosic membranes causes complement activation and generates complement products that can activate leukocytes. Leukocyte activation is associated with changes in the expression of adhesion receptors that are responsible for leukocyte sequestration and leukopenia. Himmelfarb et al also performed adhesion assays of leukocytes on monolayers of human umbilical endothelial cells [86]; the ability to adhere leukocytes with the "high Mac-1, low L-selectin" phenotype, harvested from patients during dialysis, was dramatically decreased. This intriguing finding may be explained by the rapid dialysis-induced transformation of granulocytes into a "refractory" post-activation state, in which shedding of L-selectin caused by activation [11] prevents adhesion. Preliminary results suggest that other changes in surface adhesion molecules caused by dialysis, such as reduced ICAM-1 expression [87], account for the loss of the adhesive capacity of leukocytes and thus may contribute to the increased susceptibility to infection of dialysis patients.

Another leukocyte dysfunction caused by hemodialysis is degranulation of peripheral blood neutrophils. Degranulation occurs mainly, but not exclusively, with cuprophane membranes and

is an untoward phenomenon that participates in the pathogenesis of the catabolic state and of the carpal-tunnel syndrome, which are frequently observed in dialysis patients [88, 89]. Recently, Cheung et al [89] have shown that products generated by the contact of plasma with the cuprophane membranes are responsible for degranulation. These products have not been precisely identified but are in part complement dependent, in part complement independent. In any case, they act on leukocytes through a common pathway that consists of $beta_2$ integrins. In fact, unlike normal leukocytes, those harvested from a patient with a congenital deficiency of $beta_2$ integrins do not degranulate when incubated with plasma and cuprophane membrane.

In summary, the present information indicates that adhesion molecules mediate leukocyte dysfunctions caused by hemodialysis. Therefore the effects of different dialysis membranes on leukocyte adhesion receptors should be taken into account as an important index of biocompatibility.

Adhesion molecules and other renal disorders

Acute renal failure. Obstruction of renal tubules by desquamated cells and debris plays an important pathogenic role in post-ischemic and nephrotoxic acute renal failure [90, 91]. Recent studies have shown that the majority of cells desquamated after renal insults are viable, suggesting that active cell aggregation may contribute to the development of tubular obstruction [92]. In addition, it has been found that oxidant stress to renal epithelia results in the disruption of focal contacts, the disappearance of talin from the basal cell surface (talin is a cytoskeleton protein connected with the intracellular part of integrin receptors), and a redistribution of the $alpha_3$ integrin subunit from its predominantly basal location to the apical cell surface. These changes are associated with decreased adhesion of tubular cells to wells coated with type IV collagen, laminin, fibronectin and vitronectin [93]. The ectopically expressed apical integrins, however, are functionally competent and capable of mediating cell-cell adhesion in cell suspensions [94]. Based on the above observations, the following scenario has been hypothesized for the development of tubular obstruction secondary to tubular cell insult: the loss of basolateral expression of integrins is responsible for the detachment of cells from the matrix, whereas the expression of integrin receptors on the apical surface accounts for facilitated cell-cell adhesion and eventual tubular obstruction [95] This hypothesis is supported by the observation that infusion of an integrin inhibitor in rats subjected to renal ischemia prevents a rise in intratubular pressure, the pathophysiological marker of tubular obstruction [94].

Renal cysts. Renal cyst formation derives from abnormal tubular cell proliferation and is accompanied by abnormalities in the synthesis of matrix by cyst-lining cells [96] and changes in epithelial polarity [97]. It is known that epithelial cells are responsible for the composition of their basement membranes, and the basement membrane in turn modulates cell movement, division, differentiation and polarity. As discussed previously, tubule cell-matrix interactions are mediated by adhesion receptors, mainly of the integrin family. It is not surprising, therefore, that preliminary results indicate that members of the $beta_1$ integrin family are involved in the dysregulation of the epithelial polarity that occurs in a model of *in vitro* cyst formation [98]. A role for attenuated expression of epithelial cell adhesion molecules has been suggested in a murine model of polycystic kidney disease [99].

Renal cell carcinoma. Integrin distribution has been studied in renal cell carcinomas of various grades of malignancy [100]. The $alpha_3$ subunit was detected in all tumors regardless of the grade of malignancy. This finding is interesting because renal cell carcinomas are believed to originate from both proximal and distal tubular cells, while in normal kidney $alpha_3$ expression is restricted to the distal tubule. The $alpha_6$ subunit was expressed in low-grade malignancies, in which it coaligned with basement membrane components expressed in the matrix. In contrast, the $alpha_6$ subunit was absent in G3 carcinomas, reflecting a complete disruption of cell-basement membrane interaction and, possibly, metastatic invasiveness. Noteworthy was the *de novo* expression of $alpha_v$ chain, which is not present in normal kidney and is correlated with the degree of malignancy. In summary, these results indicate that changes in integrin expression are associated with malignant transformation of cells in renal carcinoma and may play a role in their metastatic diffusion.

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